INFECTION AND IMMUNITY, June 1987, p. 1436–1440
0019-9567/87/061436-05$02.00/0

Vol. 55, No. 6

Enhanced Resistance of Mice to Bacterial Infection Induced by Recombinant Human Interleukin-1a

YUKIO OZAKI,[1]* TATSUYA OHASHI,[1] AKIRA MINAMI,[2] AND SHIN-ICHI NAKAMURA[2]

First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo,[1] and Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Osaka,[2] Japan

Received 26 November 1986/Accepted 16 March 1987

The effect of recombinant human interleukin-1a on the survival rate of Std-ddY male mice systemically infected with *Pseudomonas aeruginosa* 12 or *Klebsiella pneumoniae* P-5709 was evaluated. In *P. aeruginosa* infection, interleukin-1a given intramuscularly twice, 3 days and 1 day before inoculation of bacteria, most effectively protected animals from death due to infection. The effect was dose dependent, with a maximum survival rate of 92.5% at 10 μg per mouse, while only 8.3% of the control group survived until the end of the observation period. The 50% effective dose of interleukin-1a was 0.261 μg per mouse. In *K. pneumoniae* infection, interleukin-1a given intramuscularly twice, simultaneously with and 1 day after the inoculation of bacteria, was most effective. The protective effect of interleukin-1a was again dose dependent and was generally more marked than in *P. aeruginosa* infection. The 50% effective dose was 0.034 μg per mouse. In both infections, there was no significant increase in the survival rates of animals injected with human albumin or heat-inactivated interleukin-1a. These observations raise the possibility that human interleukin-1a could serve as a therapeutic tool for patients with bacterial infections.

Interleukin-1 (IL-1), which is thought to be identical to endogenous pyrogen, is a cytokine produced mainly by mononuclear phagocytes (5, 7, 16). It has a wide range of biologic activities; it induces both fever (1) and the production of acute-phase reactants (19; C. A. Dinarello, K. P. W. J. McAdam, and L. J. Rosenwasser, Clin. Res. 29:484, 1981). It attracts phagocytes to inflammatory sites (14, 20) and activates a variety of phagocyte functions (11, 12). Elevated body temperature caused by IL-1 and IL-1 itself have been shown to potentiate antibody production by B cells (2, 13). Some of the acute-phase reactants act as opsonins to promote ingestion of invading organisms by phagocytes (18). Although the significance of IL-1 biologic activities has not been completely clarified, together these findings suggest that IL-1 is indeed produced to facilitate the eradication of invading organisms as soon as possible.

In 1975, Kampschmidt and Pulliam (8) demonstrated that crude preparations of leukocytic endogenous mediator, which is now considered to fall into the category of IL-1, increases the survival rate of infected animals. Although their findings suggest that IL-1 actually works in vivo to protect animals from fatal bacterial infection, the limited degree of purification did not eliminate the possibility that other proteins derived from neutrophils might contribute to these effects. In the present study, we demonstrate, using purified recombinant human IL-1a, that the IL-1a significantly increased in a dose-dependent manner the survival rate of experimentally infected animals.

MATERIALS AND METHODS

Materials. Recombinant human IL-1a was produced in *Escherichia coli* and purified to homogeneity from the cell extract by salting out with ammonium sulfate, DEAE-Sepharose CL-6B column chromatography, and gel filtration on a Sephacryl S-200 column (3). Purified IL-1a showed a single band with a molecular weight of about 18,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). Purified IL-1a consisted of the C-terminal 159 amino acids of the human IL-1a precursor, and the pI was about pH 5.3. Its lymphocyte-activating factor activity, determined by the augmentation of thymocyte proliferation (4), was approximately 2×10^7 U/mg of protein. The endotoxin content of the IL-1a preparation was less than 0.08 ng/mg of protein. Heat-inactivated IL-1a was prepared by heating it at 90°C for 30 min.

Assessment of therapeutic activity. The in vivo therapeutic effect of IL-1a was assessed in systemically infected groups of eight or more Std-ddY mice weighing approximately 20 g each. Systemic infection was produced by inoculating male mice intraperitoneally with *Pseudomonas aeruginosa* 12 or *Klebsiella pneumoniae* P-5709 suspended in 0.25 ml of TryptoSoy broth (Eiken Co., Tokyo, Japan). The number of cells inoculated per mouse was 3×10^6 (three times the 50% lethal dose) for *P. aeruginosa* and 20 (four times the 50% lethal dose) for *K. pneumoniae*. These inocula were prepared by diluting fresh cultures with the broth to an appropriate optical density. The dose of IL-1a, dissolved in 100 μl of 5 mM phosphate-buffered saline (pH 7.4) containing 0.1% gelatin, was injected intramuscularly into mice at a time before or after the inoculation of bacteria. Some groups received intramuscular injections of human albumin (Sigma Chemical Co., St. Louis, Mo.) at the same protein concentration and on the same schedule as IL-1a. The control group of infected mice received no treatment.

Therapeutic effects, expressed as the percentage of survivors relative to the total number infected, were evaluated 1 week postinoculation of *P. aeruginosa* and 2 weeks postinoculation of *K. pneumoniae*. The significance of therapeutic effects of IL-1a was determined by the chi-square test and the mean effective dose (ED_{50}) was calculated by probit analysis (15).

RESULTS

Systemic infection with *P. aeruginosa*. The control group of mice injected with 3×10^6 cells showed a mortality rate of

* Corresponding author.

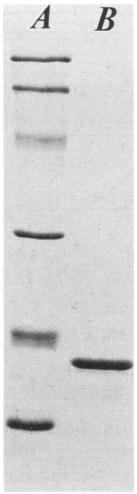

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified human IL-1a produced in *E. coli*. Samples were dissolved in 1% sodium dodecyl sulfate solution containing 5% β-mercaptoethanol and electrophoresed on a 12.5% polyacrylamide gel which was stained with Coomassie brilliant blue. Molecular weight standards in lane A (from top to bottom): phosphorylase *b*, 92,500; bovine serum albumin, 66,200; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; lysozyme, 14,400. Lane B, 5 μg of purified human IL-1a.

approximately 80% within 24 h of inoculation, and more than 90% succumbed within 2 days. In a preliminary study, we found that double administration of IL-1a was far more effective than single administration was. Thereafter, the effects of double administration of IL-1a were evaluated. The results of a preliminary study in which IL-1a was administered twice at various intervals before or simultaneously with bacterial inoculation are shown in Table 1. IL-1a was not injected after inoculation, since the control group almost totally succumbed within 24 h. The survival rate was highest when IL-1a was injected twice, 3 days and 1 day before bacterial inoculation (Table 1). In all experiments thereafter, this IL-1a administration schedule was used.

TABLE 1. Administration schedule for IL-1a and the survival rate of mice with *P. aeruginosa* infection[a]

Schedule	Dose (μg/mouse)	No. of mice (survivor/tested)	% Survival
−1d, 0h	3	5/16	31.3
	1	4/16	25.0
	0.3	2/16	12.5
	0.1	3/16	18.8
−2d, 0h	3	0/8	0
	1	1/8	12.5
	0.3	0/8	0
	0.1	1/8	12.5
−3d, −1d	10	6/8	75.0
	3	17/24	70.8
	1	14/32	43.8
	0.3	6/24	25.0
	0.1	11/32	34.4
−3d, −2d	3	4/8	50.0
	1	1/8	12.5
	0.3	3/8	37.5
	0.1	1/8	12.5

[a] IL-1a was administered to mice twice, at the intervals and times indicated. −d, Day(s) before bacterial inoculation; 0h, simultaneous inoculations with IL-1a and bacteria. The control group received no treatment; 3 of 32 infected control mice survived (survival rate, 9.4%). All mice were injected intraperitoneally with 3×10^6 *P. aeruginosa* cells, and the mortality rate was observed 7 days after inoculation.

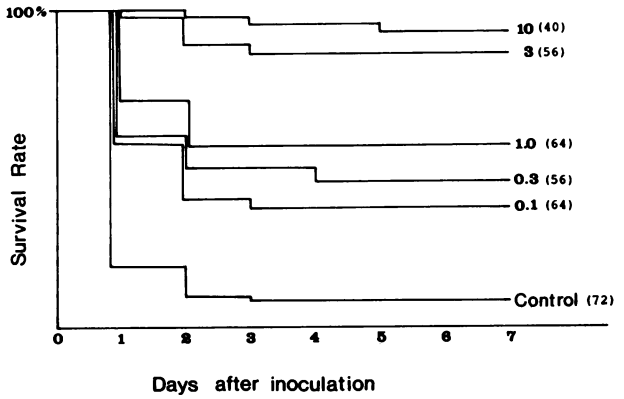

FIG. 2. Survival studies on mice receiving 3×10^6 *P. aeruginosa* cells. The indicated doses of IL-1a (micrograms per mouse) were injected intramuscularly twice, 3 days and 1 day before inoculation of *P. aeruginosa*. The control group received no treatment. The mice were then injected intraperitoneally with 3×10^6 *P. aeruginosa* cells, and the mortality rate was observed for 7 days after bacterial inoculation. The number of animals in each group is shown in parentheses.

The survival curves of mice receiving two injections of IL-1a, at various concentrations at 3 days and 1 day before bacterial inoculation, are shown in Fig. 2. Most mice that were alive 2 days after inoculation survived until the end of the observation period (7 days after inoculation), and all were healthy. IL-1a increased the survival rate in a dose-dependent manner, and the group receiving two injections of 10 μg each showed a survival rate of 92.5%, while only 8.3% of the control group survived until the end of the observation period. The groups receiving intramuscular injections of human albumin or heat-inactivated IL-1a (3 μg) on the same schedule showed no significant increase in survival rate. There were no apparent toxic effects of IL-1a, except in the group receiving two shots of 10 μg each; these mice showed a moderate loss of body weight and fluffing up of their fur for a few days after the second IL-1a injection. Although the therapeutic effect decreased with a lower dose, the survival rate even at 0.01 μg per mouse was significantly higher than that of the control group ($P < 0.01$) (Table 2). The ED_{50} for IL-1a in *P. aeruginosa* infection was 0.261 μg per mouse, with a 95% confidence range of 0.05 to 1.31 μg per mouse.

Systemic infection with *K. pneumoniae*. In contrast to the rapid course of infection caused by 3×10^6 *P. aeruginosa* cells, systemic infection induced by 20 *K. pneumoniae* cells developed more slowly. On day 1 after inoculation, no mortality was observed, although from days 2 to 6 the total number of living individuals decreased gradually. Almost all mice that were alive on day 6 after inoculation survived throughout the 14-day observation period.

Since IL-1a proved to be more effective against *P. aeruginosa* infection when administered twice, the effects of mostly dual doses of IL-1a were also evaluated in *K. pneumoniae* infection. The effects of IL-1a injected twice at the same concentration, but at various intervals and times are shown in Table 3. In sharp contrast to the effects of IL-1a

TABLE 2. Effect of IL-1a on the survival rate of mice with *P. aeruginosa* infection[a]

Agent	Dose (μg/mouse)	No. of mice (survivor/tested)	% Survival
IL-1a	10	37/40	92.5
	3	48/56	85.7
	1	36/64	56.3
	0.3	26/57	46.4
	0.1	24/64	37.5
	0.03	20/40	50.0
	0.01	14/40	32.5
Human albumin	10	2/16	12.5
	3	1/16	6.3
	1	3/16	18.8
	0.3	1/8	12.5
	0.1	0/8	0
Heat-inactivated IL-1a	3	2/24	8.3

[a] Doses of IL-1a were injected intramuscularly twice, 3 days and 1 day before *P. aeruginosa* inoculation. Some groups received two intramuscular injections of either human albumin or heat-inactivated IL-1a on the same schedule as IL-1a. The control group received no treatment; 6 of 72 infected control mice survived (survival rate, 8.3%). All mice were injected intraperitoneally with 3×10^6 *P. aeruginosa* cells, and the survival rate was observed 7 days after bacterial inoculation. For IL-1a at all doses, the survival rate was significantly higher ($P < 0.01$) than that of the control mice, and the ED_{50} was 0.261 (95% confidence range, 0.05 to 1.31).

on systemic infection induced by *P. aeruginosa*, medication schedules that included a simultaneous injection of IL-1a with inoculation of bacteria were more effective than injections at 3 days and 1 day before bacterial inoculation. IL-1a most effectively increased the survival rate of infected mice when given twice, simultaneously with and 24 h after inoculation. Further evaluation of the effects of IL-1a was made by using this medication schedule. The survival curves for infected mice that received two intramuscular injections of IL-1a at various doses simultaneously with and 1 day after inoculation are shown in Fig. 3. Regardless of the IL-1a doses, almost all mice that were alive on day 6 after inoculation survived the 14-day observation period in good condition. IL-1a increased the survival rate of infected mice in a dose-dependent manner, with an optimal dose of 3 μg per mouse. Groups receiving IL-1a at doses higher than 1 μg per mouse showed survival rates of more than 90%, while only 6.3% of the control group survived until the end of the observation period. IL-1a, even at a dose of 0.01 μg per mouse, significantly elevated the survival rate above that of the control group ($P < 0.01$). Groups receiving intramuscular injections of human albumin or heat-inactivated IL-1a (3 μg) on the same schedule as that of native IL-1a showed no significant improvement in survival rate. The ED_{50} was 0.034 μg per mouse, with a 95% confidence range of 0.01 to 0.13 μg per mouse (Table 4).

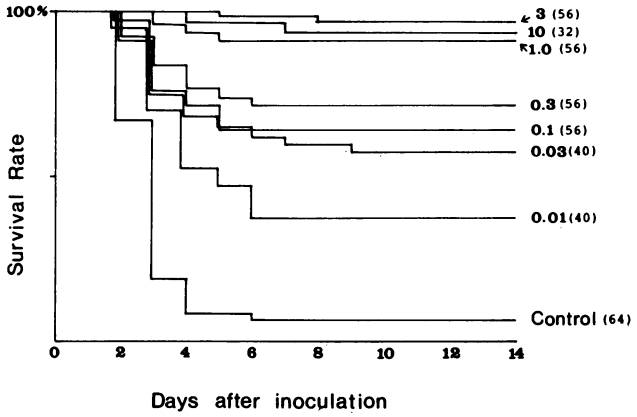

FIG. 3. Survival studies on mice receiving 20 *K. pneumoniae* cells. The indicated doses of IL-1a (micrograms per mouse) were injected intramuscularly twice, simultaneously with and 24 h after inoculation of 20 *K. pneumonie* cells. The control group was intraperitoneally injected with organisms without subsequent injections of IL-1a. The mortality rate was observed for 14 days after inoculation of the bacteria. The number of animals in each group is shown in parentheses.

TABLE 3. Administration schedule for IL-1a and the survival rate of mice with *K. pneumoniae* infection[a]

Schedule	Dose (μg/mouse)	No. of mice (survivor/tested)	% Survival
−3d, −1d	3	5/24	20.8
	1	4/24	16.7
	0.3	2/24	8.3
	0.1	6/24	25.0
−2d, 0h	3	4/8	50.0
	1	6/8	75
	0.3	1/8	12.5
	0.1	0/8	0
−1d, 0h	3	21/24	87.5
	1	15/24	62.5
	0.3	7/24	29.2
	0.1	1/24	4.2
0h, 1d	3	23/24	95.8
	1	20/24	83.3
	0.3	12/24	50.0
	0.1	11/24	45.8

[a] All mice were injected intraperitoneally with 20 *K. pneumoniae cells*; IL-1a was administered twice at the doses, intervals, and times as indicated. −d, Day(s) before bacterial inoculation; 0h, simultaneous inoculations with IL-1a and bacteria. The control group received no treatment; 2 of 24 infected control mice survived (survival rate, 8.3%). The mortality rate was observed 14 days after inoculation.

DISCUSSION

The results of this study clearly demonstrate that highly purified IL-1a produced by recombinant DNA technology increased the survival rate of infected mice in a dose-dependent manner. The ineffectiveness of human albumin and heat-inactivated IL-1a given at the same protein concentration on the same schedule as that of native IL-1a suggests that the protective effect of IL-1a against infection was due to specific biological activities.

In terms of the dose required, IL-1a was more effective in protecting animals against *K. pneumoniae* than against *P. aeruginosa*. However, the major differences in the effects of IL-1a on systemic infection induced by these two organisms appear to lie in the medication schedules. IL-1a was most effective against *P. aeruginosa* when given 3 days and 1 day before inoculation of bacteria, while IL-1a showed better protective effects against *K. pneumoniae* infection when given simultaneously with and 24 h after inoculation. Several mechanisms could be postulated that may account for this discrepancy. First, the latency period before the appearance of the protective effect of IL-1a should be discussed. Infection in mice caused by 3×10^6 *P. aeruginosa* cells showed a

TABLE 4. Effect of IL-1a on the survival rate of mice with *K. pneumoniae* infection[a]

Agent	Dose (μg/mouse)	No. of mice (survivor/tested)	% Survival
IL-1a	10	30/32	93.8
	3	54/56	96.4
	1	51/56	91.1
	0.3	40/56	71.4
	0.1	36/56	64.3
	0.03	23/40	57.5
	0.01	15/40	37.5
Human albumin	10	4/24	16.7
	3	4/24	16.7
	1	3/24	12.5
	0.3	0/8	0
	0.1	0/8	0
Heat-inactivated IL-1a	3	0/24	0

[a] The mice were injected intraperitoneally with 20 *K. pneumoniae* cells; IL-1a was administered intramuscularly twice, simultaneously with and 24 h after inoculation of the bacteria. Some groups received human serum albumin or heat-inactivated IL-1a on the same schedule as IL-1a. The control group received no treatment; 4 of 64 infected control mice survived (survival rate, 6.3%). The survival rate was observed 14 days after bacterial inoculation. For IL-1a at all doses, the survival rate was significantly higher ($P < 0.01$) than that of the control mice, and the ED_{50} was 0.034 (95% confidence range, 0.01 to 0.13).

rapid course, in which most animals died within 24 h after inoculation. An agent that has a direct inhibitory or cytotoxic effect on bacteria might save the animals in this type of infection, while other agents that exert a protective effect indirectly, that is, by potentiating a host defense mechanism, might fail to save them if the infection proceeds so rapidly. IL-1a, even at a concentration of 100 μg/ml, had no direct inhibitory effect on bacteria (data not shown), and if we postulate that a certain delay is required before IL-1a-recruited protective factors appear in the host, we may well expect IL-1a to be ineffective in fulminant infections when given at the time of or shortly after bacterial inoculation. In our clinical experience, we have very rarely encountered cases of infection with such a rapid course, and the results of our study on *P. aeruginosa*-infected mice may well be interpreted as suggesting a possible therapeutic role for IL-1a in patients suffering from bacterial infections. Our findings that IL-1a injected at the time of and shortly after inoculation was effective against *K. pneumoniae* infection, which showed a more prolonged course, appear to support this hypothesis.

The high protective effects of prior administration of IL-1a against *P. aeruginosa* infection and its lower effectiveness against *K. pneumoniae* raise another issue. We suggest that the IL-1a-induced enhancement of the defense mechanism, the essence of which is not yet completely identified, terminates within a few days after the last administration of IL-1a. If a critical time of infection (probably 24 h after inoculation with *P. aeruginosa* and 2 to 3 days after inoculation with *K. pneumoniae*) coincides with the height of host defense mechanism enhancement, then the spread of the bacterial infection may be interrupted. In this regard, IL-1a clearly differs from immunization with antigens, the protective effects of which generally appear much later and last longer. Nonetheless, our findings suggest that a multiple injection schedule for IL-1a at certain intervals could be more beneficial in clinical trials.

The differences in IL-1a efficacy when injected at various times may also be attributed to the number of bacteria injected into mice or to the mode of killing of bacteria in the body. It is well established that the phagocyte system plays an essential part in destroying bacteria (10) and that neutrophil function is potentiated in patients with bacterial infections (6, 17). Hence, it is possible that IL-1a in vivo enhances resistance of the host to bacterial infection by directly stimulating phagocyte function. Previous studies showed that enhanced phagocytosis cannot fully explain the potentiated infection resistance induced by endotoxin or by leukocytic endogenous mediator (6, 8), but it is still possible that other parameters of phagocyte function may be modulated or that there may be an increase in the production of antibodies or other factors that facilitate killing of bacteria by phagocytes. In this way, the stimulatory effect of IL-1a on phagocyte function may have protected the host against *K. pneumoniae* infection, which in the present study was caused by a relatively small number of organisms. On the other hand, the number of cells in experimental infection by *P. aeruginosa* might have been too large for the mouse phagocyte system to kill, even with the aid of IL-1a. The prior enhancement by IL-1a of defense mechanisms in addition to the enhancement of mechanisms involving phagocytes may be needed to save the host in this case.

In clinical practice, we sometimes encounter malnourished or debilitated patients who have signs of bacterial infection without elevated body temperature. Many practitioners have the view that the prognoses of these patients are generally poorer than the prognoses of those who manifest fever. In support of this observation, Keenan et al. (9) reported that infected patients who could produce endogenous pyrogens had a better survival rate than those who lacked the ability to produce them. On the basis of these findings, we believe that IL-1a will benefit patients with bacterial infections in malnourished, debilitated, or immunocompromised states. However, it remains unresolved whether the administration of an excessive amount of IL-1a has clinical merit to patients who can produce physiological levels of it in response to bacterial infection. The protective effect of IL-1a administration in infected patients who have a normal defense system is suggested by our findings that IL-1a increased the survival rate of infected mice, even though they were considered to have intact phagocyte and immune systems.

LITERATURE CITED

1. **Dinarello, C. A., and S. M. Wolff.** 1982. Molecular basis of fever in humans. Am. J. Med. **72**:799–819.
2. **Falkoff, R. J. M., A. Muraguchi, J.-X. Hong, J. L. Butler, C. A. Dinarello, and A. S. Fauci.** 1983. The effects of interleukin 1 in human B cell activation and proliferation. J. Immunol. **131**: 1801–1805.
3. **Furutani, Y., H. Nomura, M. Notake, M. Yamayoshi, M. Ohue, J. Yamagishi, T. Furuta, M. Yamada, and S. Nakamura.** 1985. The production of human interleukin 1 by recombinant DNA technology. Proc. Jpn. Soc. Immunol. **15**:380 (In Japanese.).
4. **Furutani, Y., M. Notake, M. Yamayoshi, J. Yamagishi, H. Nomura, M. Ohue, R. Furuta, T. Fukui, M. Yamada, and S. Nakamura.** 1985. Cloning and characterization of the cDNAs for human and rabbit interleukin-1 precursor. Nucleic Acids Res. **13**:5869–5882.
5. **Hanson, D. F., and P. A. Murphy.** 1984. Demonstration of interleukin 1 activity in apparently homogeneous specimens of the pI 5 form of rabbit endogenous pyrogen. Infect. Immun. **45**:483–490.
6. **Hill, H. R., W. J. Warwick, B. S. Dettloff, and P. G. Quie.** 1974.

Neutrophil granulocyte function in patients with pulmonary infection. J. Pediatr. **84**:55–58.
7. **Kampschmidt, R. F.** 1984. Infection, inflammation, and interleukin 1. Lymphokine Res. **2**:97–110.
8. **Kampschmidt, R. F., and L. A. Pulliam.** 1975. Stimulation of antimicrobial activity in the rat with leukocytic endogenous mediator. RES J. Reticuloendothel. Soc. **17**:162–169.
9. **Keenan, R. A., L. L. Moldawer, R. D. Yang, I. Kawamura, G. L. Blackburn, and B. R. Bistrian.** 1982. An altered response by peripheral leukocytes to synthesize or release leukocyte endogenous mediator in critically ill, protein-malnourished patients. J. Lab. Clin. Med. **6**:844–857.
10. **Klebanoff, S. J., and R. A. Clark.** 1978. Antimicrobial systems, p. 409–488. *In* S. J. Klebanoff (ed.), The neutrophils: function and clinical disorders. Elsevier/North-Holland Publishing Co., Amsterdam.
11. **Klempner, M. S., and C. A. Dinarello.** Stimulation of neutrophil oxygen dependent metabolism by human leukocytic pyrogen. J. Clin. Invest. **64**:996–1002.
12. **Klempner, M. S., C. A. Dinarello, and J. I. Gallin.** 1978. Human leukocytic pyrogen induces release of specific granule contents from human neutrophils. J. Clin. Invest. **61**:1330–1336.
13. **Lipsky, P. E., P. A. Thompson, J. L. Rosenwasser, and C. A. Dinarello.** 1983. The role of interleukin 1 in human B cell activation: inhibition of B cell proliferation and the generation of immunoglobulin-secreting cells by an antibody against human leukocytic pyrogen. J. Immunol. **130**:2708–2714.
14. **Luger, T. A., J. A. Charon, M. Colot, M. Micksche, and J. J. Oppenheim.** 1983. Chemotactic properties of partially purified human epidermal cell-derived thymocyte activating factor (ETAF) for polymorphonuclear and mononuclear cells. J. Immunol. **13**:816–820.
15. **Miller, L. C., and M. L. Tainter.** 1944. Estimation of ED_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. Med. **57**:261–264.
16. **Murphy, P. A., P. L. Simon, and W. F. Willoghby.** 1980. Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte-activating factors made by rabbit alveolar macrophages. J. Immunol. **124**:2498–2501.
17. **Ozaki, Y., T. Ohashi, and Y. Niwa.** 1986. Oxygen radical production by neutrophils from patients with bacterial infection and rheumatoid arthritis: measurement of hydrogen peroxide may most accurately represent enhancement of oxygen radical production during infection. Inflammation **10**:119–130.
18. **Pepys, M. B., and M. L. Baltz.** 1983. Acute-phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. **34**:141–212.
19. **Ramadori, G., J. D. Sipe, C. A. Dinarello, S. B. Mizel, and H. R. Colten.** 1985. Pretranslational modulation of acute-phase hepatic protein synthesized by murine recombinant interleukin-1 and purified human IL-1. J. Exp. Med. **162**:930–942.
20. **Saunder, D. N., N. L. Mounessa, S. I. Katz, C. A. Dinarello, and J. I. Gallin.** Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. J. Immunol. **132**:828–832.